

Skin Test Results but not Serology Reflect Immediate Type Respiratory Sensitivity: A Study Performed with Recombinant Allergen Molecules

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The diagnosis of type I allergy, an IgE-antibody-mediated hypersensitivity disease affecting more than 25% of the population, is based on the measurement of allergen-specific serum IgE levels and provocation testing. Whether the determination of allergen-specific serum IgE levels can replace *in vivo* provocation testing for allergy diagnosis is a controversial issue. We used purified recombinant timothy grass and birch pollen allergens to compare by skin prick and nasal provocation testing as well as by serology *in vivo* sensitivity with antibody-binding capacity in 24 pollen allergic patients and eight control individuals. Results from biologic tests were correlated with each other and with allergen-specific IgE and IgG₁₋₄ levels. IgE-reactive allergens induced immediate skin and nasal reactions, but the intensity of the allergic tissue reactions was not correlated with either the

levels of allergen-specific IgE or the levels of allergen-specific IgG antibodies. Less frequently detected allergens with low IgE-binding capacity were able to induce strong allergic reactions comparable to those caused by major allergens with high IgE-binding capacity. In contrast, skin test and nasal provocation results were significantly correlated ($r = 0.63$, $p < 0.01$). Our study thus demonstrates on a molecular level that skin testing provides a better reflection of immediate type respiratory sensitivity than serologic measurements. These results have implications for allergy diagnosis and, in particular, for the selection of relevant allergen components for specific immunotherapy. **Key words:** diagnosis/IgE/nasal provocation test/recombinant allergen/rhinomanometry/skin prick test/type I allergy. *J Invest Dermatol* 117:848–851, 2001

The immediate symptoms of type I allergy result from the allergen-induced crosslinking of effector cell (e.g., mast cell, basophil) bound IgE antibodies, which leads to the release of histamine and other inflammatory mediators (e.g., leukotrienes) (Beaven and Metzger, 1993). FcεRI represents the critical structure for the activation of effector cells by IgE–allergen complexes and it is well established that elevated serum IgE levels lead to an upregulation of FcεRI expression (MacGlashan *et al*, 1998, 1999). The question whether the levels of specific IgE antibodies in serum can act as reliable predictive markers for clinical sensitivity, however, represents a controversial issue. On the one hand, several clinical studies indicated no precise correlation between serum IgE levels and clinical symptoms (Pelikan, 1983; Bousquet *et al*, 1987; Rasanen *et al*, 1994). On the other hand, it is well established that patients exhibit increased sensitivity to allergen provocation after seasonal rises

of specific IgE levels (Henderson *et al*, 1975; Naclerio *et al*, 1997).

Studies regarding the association of serum IgE reactivity and allergenic activity have been performed mainly with allergen extracts representing complex and variable mixtures of allergenic and nonallergenic components. In this study we used defined recombinant allergen molecules instead of allergen extracts to reinvestigate the association of allergen-specific IgE serum levels with skin and nasal sensitivity.

Two recombinant tree pollen allergens [rBet v 1 (Breiteneder *et al*, 1989) and rBet v 2 (Valenta *et al*, 1991)] and three recombinant grass pollen allergens [rPhl p 1 (Laffer *et al*, 1994), rPhl p 2 (Dolecek *et al*, 1993), rPhl p 5 (Vrtala *et al*, 1993)] were used for *in vivo* testing (nasal provocation test, skin prick test) and quantitative *in vitro* measurement of specific antibody responses (IgE and IgG subclass levels).

In parallel we determined symptoms of allergen-induced rhinitis by active anterior rhinomanometry. Furthermore, immediate type skin reactions to recombinant allergens were measured by skin prick testing. Results from serology and *in vivo* provocation testing to defined recombinant allergen molecules were correlated. We discuss possible mechanisms for our findings that allergens with high as well as with low IgE-binding capacity induced strong allergic reactions and that results from both *in vivo* tests (nasal provocation and skin prick testing), but not from *in vitro* and *in vivo* tests, were correlated.

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Furthermore we discuss our findings regarding the possible application of recombinant allergens for component-resolved skin test diagnosis and specific immunotherapy.

MATERIALS AND METHODS

Patients and control individuals Twenty-four tree and/or grass pollen allergic patients were included in the study. Patients were selected on the basis of a positive case history indicative of tree and/or grass pollen allergy [presence of at least one of the symptoms (rhinitis, conjunctivitis, or asthma)] during the tree and/or grass pollen season and elevated serum IgE levels to birch and/or grass pollen extract as determined by use of the Pharmacia CAP system (Pharmacia, Uppsala, Sweden).

At the time of the study all patients had stable lung function and there was no evidence of airway infection. Patients who had received corticosteroid therapy or antihistamines or who suffered from atopic dermatitis were excluded from the study.

Five nonallergic individuals and three allergic individuals without sensitization to the tested allergens were included as controls.

Study design The serum IgE levels specific for recombinant allergens (rPhl p 1, rPhl p 2, rPhl p 5, rBet v 1, and rBet v 2) were determined by quantitative CAP-RAST measurements (Pharmacia; **Table I**). According to the CAP-RAST determinations we selected for each patient the recombinant allergen yielding the highest and that yielding the lowest specific serum IgE levels for *in vivo* provocation testing (i.e., nasal provocation and skin prick testing). For control purposes, eight individuals were subjected to provocation testing with three allergens against which they had no IgE antibodies.

The study was conducted outside the tree and grass pollen season (winter time) on three different days with a 4 wk interval between study days. On each study day serum samples were collected, skin prick tests were performed with both selected recombinant allergens, and nasal provocation testing was done with one of the two selected recombinant allergens or placebo, respectively. Nasal provocation tests were performed in a double-blinded and randomized manner.

The study was approved by the Ethical Committee of the Faculty of Medicine, University of Vienna, Austria. Each patient and control person gave written informed consent.

Recombinant allergens Purified recombinant timothy grass pollen allergens (rPhl p 1, rPhl p 2, rPhl p 5) and recombinant birch pollen allergens (rBet v 1 and rBet v 2) that had been expressed in *Escherichia coli* (Susani *et al*, 1995; Vrtala *et al*, 1996; Hoffmann-Sommergruber *et al*, 1997) were obtained from Biomay (Vienna, Austria).

Determination of total IgE, specific IgE, and IgG subclass levels Total IgE and IgE levels to birch and timothy grass as well as to rPhl p 1, rPhl p 2, rPhl p 5, rBet v 1, and rBet v 2 were determined by quantitative CAP-RAST measurements (Pharmacia).

In order to visualize a possible influence of allergen-specific IgG antibodies on IgE binding, IgE enzyme-linked immunosorbent assay (ELISA) determinations were performed using low allergen concentrations for coating of the plates (25 ng per well). The detection of allergen-specific IgE antibody reactivity by ELISA was performed as previously described (Laffer *et al*, 1996). In addition, IgG subclass levels to purified recombinant allergens were measured by ELISA (Vrtala *et al*, 1996).

Skin prick testing Skin prick tests were performed with recombinant allergens diluted in 0.9% sodium chloride solution (5 µg per ml and 20 µg per ml). Sterile 0.9% sodium chloride solution and histamine hydrochloride (ALK, Hørsholm, Denmark) were used for control purposes. Twenty microliter aliquots of the test solutions were placed on the patients' forearms with a distance of more than 3 cm between individual application points. Reactions were recorded 20 min after testing by photography of patients' forearms and by transferring the ballpoint pen surrounded weal reaction with a scotch tape to paper. The maximal longitudinal and transversal diameter of the weal was measured and the mean diameter of the weal was calculated according to the formula $(D_1 + D_2)/2$. D_1 represents the maximal longitudinal and D_2 the maximal transversal diameter.

Nasal provocation testing Nasal provocation tests were performed with two selected recombinant allergens or placebo on one of three different study days, respectively. To avoid unspecific nasal reactions, a time interval of 4 wk was left between different study days.

Table I. Comparison of average results obtained by serology, skin testing and nasal provocation with different recombinant allergens in the number of patients (n) tested

	IgE (kUA per liter)	Nasal provocation and rhinomanometry (points)	Skin prick test (mean diameter, mm)
Phl p 1	24.2	19.6	5.0 (n = 5)
Phl p 2	4.47	46.3	14.0 (n = 11)
Phl p 5	22.4	41.8	11.8 (n = 19)
Bet v 1	22.5	50.5	14.2 (n = 5)
Bet v 2	3.44	39.1	11.1 (n = 8)

On each study day, the baseline level of nasal parameters (nasal flow, resistance, and flow decrease) was established by active anterior rhinomanometry without administration of test substances (Bachert, 1997; Melillo *et al*, 1997). Next, 0.9% sodium chloride solution was applied to rule out any nonspecific hyperreactivity.

Thereafter, the patient received increasing doses of allergen (5 µg per ml, 10 µg per ml, 20 µg per ml, 40 µg per ml) or placebo in 15 min intervals. Allergen solutions were administered into one nostril using a metered pump delivering 15 µl per puff. During application of test solutions, the patient had to hold his or her breath in full inspiration to avoid bronchial provocation.

Evaluations were performed 15 min after each provocation by measuring of objective and subjective parameters. Objective parameters were (i) the number of sneezes, (ii) the weight of nasal secretions (weighing handkerchiefs), and (iii) the reduction in nasal airflow determined by active anterior rhinomanometry. Subjective parameters comprised (i) itching, (ii) sneezing, (iii) nasal secretion, (iv) nasal congestion, and (v) symptoms in other organs (e.g., itching of ears).

For each of the objective and subjective parameters 0–4 points could be achieved after administration of each of the four allergen concentrations. The points achieved after testing of all four allergen concentrations were added to yield the total score. When a nasal airflow reduction of 60% was exceeded, testing was stopped and full points were given for the remaining allergen concentrations.

Endpoints and statistical analysis Total scores from nasal provocation, mean weal diameters (mm), total IgE (kU per liter), specific serum IgE (kUA per liter), and IgG (optical density values) levels determined for the same allergen on the same study day were compared using Spearman regression analysis.

RESULTS

Patients with pollen-induced allergic rhinitis exhibit IgE responses of greatly varying magnitudes to individual allergen components We studied 24 grass and/or tree pollen allergic patients suffering from seasonal allergic rhinitis and eight control individuals without clinical manifestation of pollen allergy. The group of allergic patients consisted of 14 men and 10 women with a mean age of 28.4 y. Twenty-three of the 24 patients also had allergic conjunctivitis and 12 patients suffered from asthma. RAST serology (CAP, Pharmacia) showed that all 24 patients contained IgE antibodies to timothy grass pollen extract and 16 patients had IgE antibodies to birch pollen extract. All 24 patients displayed IgE antibodies to Phl p 1, 22 reacted with Phl p 2, and 22 patients contained IgE against Phl p 5. Bet v 1 was recognized by 12 of 16 birch pollen allergic patients and 10 of these patients also reacted with Bet v 2. Patients mounted widely varying specific IgE levels to the recombinant allergen components tested (rPhl p 1, 2.16–65.7 kUA per liter, average 21.0 kUA per liter; rPhl p 2, 0.40–42.3 kUA per liter, average 11.3 kUA per liter; rPhl p 5, 1.57–83.3 kUA per liter, average 21.8 kUA per liter; rBet v 1, 0.45–98.1 kUA per liter, average 29.1 kUA per liter; rBet v 2, 0.40–11.5 kUA per liter, average 4.62 kUA per liter) (**Table I**).

The allergenic activity of individual allergen components cannot be predicted by serology: allergens with low IgE-binding capacity can induce strong allergic reactions In **Fig 1** we compare allergen-specific IgE levels (x axis, kUA per

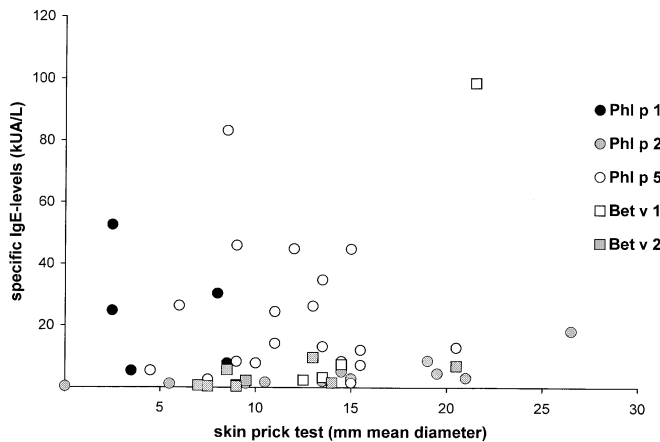


Figure 1. Relation between specific IgE levels and mean diameter of skin reactions.

liter) with the results of skin prick testing (y axis, mean weal diameters of skin reactions), and in **Fig 2** with results from nasal provocation testing (y axis, points) by scatter plotting. We found no correlation between allergen-specific IgE levels and mean weal diameters ($r = 0.1$) (**Fig 1**). Likewise, allergen-specific IgE levels and the scores achieved in nasal provocation tests were not correlated ($r = 0.2$) (**Fig 2**).

The lack of correlation between allergen-specific IgE levels and biologic test results is also emphasized by the observations (i) that the highest average IgE levels were directed against recombinant timothy grass pollen allergen, rPhl p 1 (24.2 kUA per liter), which induced the weakest average biologic reactions in both provocation tests (19.6 points, 5 mm MD) (**Table I**), and (ii) that the two allergens with low average IgE-binding capacity (rPhl p 2, 4.47 kUA per liter, 14.0 mm MD, 46.3 points; rBet v 2, 3.44 kUA per liter, 11.1 mm MD, 39.1 points) induced biologic reactions comparable to those induced by allergens with high average IgE-binding capacity (rPhl p 5, 22.4 kUA per liter, 11.8 mm MD, 41.8 points; rBet v 1, 22.5 kUA per liter, 14.2 mm MD, 50.5 points) (**Table I**).

These results indicate that allergens that in quantitative IgE assays bind low levels of specific IgE can induce strong clinical symptoms.

The allergenic potency of recombinant allergens can be defined by skin prick testing as well as by nasal provocation testing: the results of both *in vivo* tests are significantly correlated Scatter plot comparison of the results obtained by skin prick testing (mean weal diameters) with those gained by nasal provocation testing (scores) revealed a strong and significant correlation ($r = 0.63$, $p < 0.01$) (**Fig 3**). Thus, the biologic activity (i.e., the potency to induce clinical symptoms) of a given allergen was equally well defined by skin prick testing and by nasal provocation testing (**Fig 3**, **Table I**). Individuals who were tested with allergens against which they contained no IgE antibodies displayed no reaction in skin prick or nasal provocation tests.

Lack of evidence that allergen-specific IgG antibodies are responsible for the discrepancy between IgE levels and biologic reactions in the tested patients The following two observations suggest that the lack of correlation between allergen-specific IgE levels and results obtained by biologic tests in our study group cannot be explained by the presence of allergen-specific IgG antibodies. (i) No correlation between allergen-specific IgG subclass levels, IgE levels, and biologic test results was observed (data not shown). (ii) When determinations of allergen-specific IgE were carried out with low concentrations of allergens allowing competition of IgG, similar results to determinations carried out in allergen excess were obtained ($r = 0.8$; data not shown).

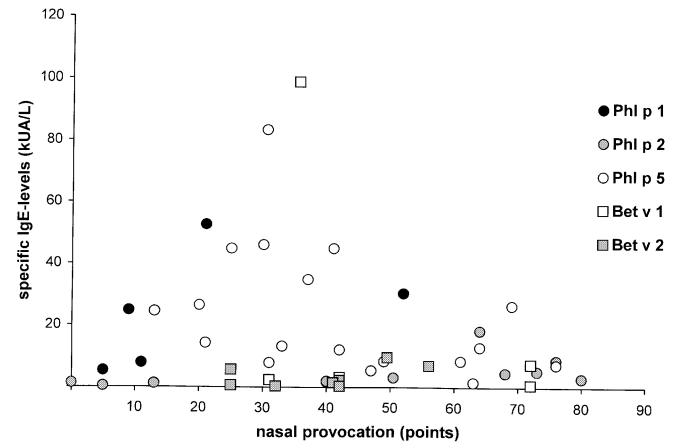


Figure 2. Relation between specific IgE levels and points obtained in nasal provocation tests.

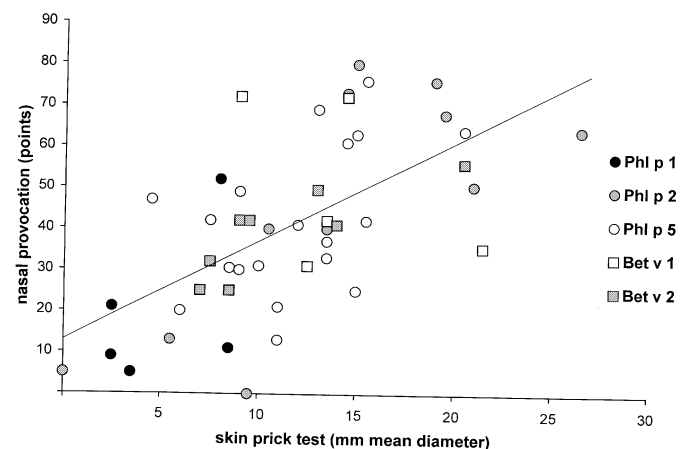


Figure 3. Relation between mean diameter of skin reactions and points obtained in nasal provocation tests.

DISCUSSION

Using recombinant pollen allergen molecules we found no correlation between the amount of allergen-specific serum IgE levels and results from nasal provocation and skin prick tests. Allergens with low IgE-binding capacity (rPhl p 2, rBet v 2) were able to induce biologic reactions comparable to those induced by allergens with high IgE-binding capacity (rPhl p 5, rBet v 1) and caused even stronger reactions than rPhl p 1, a major allergen that is recognized by more than 95% of grass pollen allergic patients. These results demonstrate that allergen extracts currently used for diagnosis and therapy contain several allergen components of which some can induce much stronger allergic reactions than others. Thus the IgE-binding capacity of a given allergen cannot be used to predict its allergenic activity.

We must therefore consider that factors other than the IgE-binding capacity of a particular allergen can influence the extent of the allergic tissue reaction induced by the molecule. Results obtained with recombinant allergen molecules in our study show that three suspected possibilities for the discrepancy between serology and clinical sensitivity are not sufficient to explain the phenomenon. The first possibility, that the number and/or sensitivity of allergic effector cells in the target organs (e.g., respiratory tract, skin) dictate the intensity of the allergic reaction, cannot explain the different responsiveness to various allergen components.

The second explanation, that allergen-specific IgG either competed with IgE or, via co-crosslinking, inactivated effector cells, is also not supported by our data. We found that allergen-specific IgG₁₋₄ levels were not correlated with allergen-specific IgE levels nor with nasal or skin sensitivity. When ELISA plates were coated with low amounts of allergens to allow competition of allergen-specific IgG with IgE binding, comparable results were obtained to the determinations performed in allergen excess, and the correlation between serology and clinical sensitivity did not improve. We must admit, however, that an experimental setup that would allow investigation of the possibility that co-crosslinking of effector cell bound IgE and IgG could have caused the discrepancy between serology and clinical sensitivity in the nose and skin has not yet been available to us.

The third assumption, that the biologic function of a particular allergen could be related to its allergenic activity in the nose or skin, cannot be supported by our results. Allergens with possible RNase (Bet v 1, Phl p 5) or protease/expansin (Phl p 1) activity in general did not induce stronger tissue reactions than allergens without enzymatic activity [e.g., Bet v 2, profilin, an actin-binding ubiquitous cytoskeletal protein (Valenta *et al*, 1991); Phl p 2, an allergen mimicking the structure of an immunoglobulin domain (De Marino *et al*, 1999)].

We therefore propose that (i) the number and/or density of IgE epitopes on a particular allergen, (ii) the affinity/avidity of IgE antibodies, (iii) the recognition of allergens by certain IgE variants and/or (iv) the structural orientation of IgE epitopes on an allergen can influence the magnitude of the effector cell response.

In summary our data show that allergic patients can exhibit a widely varying sensitivity to individual allergen components that cannot be predicted by quantitative IgE serology alone. Our finding that skin sensitivity showed a good correlation with nasal sensitivity indicates that skin testing with allergen molecules may be useful to predict the potential of allergens to induce respiratory symptoms and thus help to identify the most relevant allergen components for specific immunotherapy. New forms of component-resolved immunotherapy that are based on the use of synthetic and genetically modified allergens with reduced allergenic activity are currently being developed and can then be tailored to the patients' sensitization profile established by component-resolved allergy diagnosis (Valenta and Kraft, 2001).

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